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(54) Title: A DRUG BASED ON A SUBSTANCE P ANTAGONIST

(57) Abstract

A Substance P antagonist for use in drugs for the prophylactic or therapeutic treatment of diseases released by Substance P. The antagonist consists of (D-Pro^2, D-Trp^{7,9}) Substance P having the formula

L-Arg-D-Pro-L-Lys-L-Pro-L-Gln-L-Gln-D-Trp-L-Phe-D-Trp-L-Leu-L-Met-NH_2

A drug for the prophylactic or therapeutic treatment of inflammations, especially in the eye, is also described, said drug containing as the active substance the above-mentioned Substance P antagonist. Furthermore, there is described a meth d of prophylactically or therapeutically treating a disease which is released by Substance P, or inflammations, especially in the eye. In this method, use is made of a therapeutically active amount of (D-Pro^2, D-Trp^{7,9}) Substance P, especially in a form suitable for topical application.

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A DRUG BASED ON A SUBSTANCE P ANTAGONIST

The present invention relates to a Substance P antagonist for use in drugs for the prophylactic or therapeutic treatment of diseases released by Substance P, to a method of prophylactically or therapeutically treating such a disease, and to a drug for prophylactically or therapeutically treating inflammations. More specifically, the invention relates to a Substance P antagonist which, in relation to Substance P, has a structure in which L-proline in position 2 has been replaced by D-proline, and L-phenylalanine in position 7 and L-glycocoll in position 9 have been replaced by D-tryptophan.

The Substance P antagonist according to this invention thus is (D-Pro^2, D-Trp^{7,9}) Substance P having the formula

L-Arg-D-Pro-L-Lys-L-Pro-L-Gln-L-Gln-D-Trp-L-Phe-D-Trp-L-Leu-L-Met-NH_2

Background

In recent years, it has been shown that substances which transmit signals to the nervous system, so-called transmitter substances or transmitters, consist not only of substances related to adrenaline and acetyl choline (adrenergic and cholinergic nerves, respectively), but also of chemical compounds made up of a series of amino acids joined by peptide bonds, i.e. polypeptides or oligopeptides. In most cases, peptides alone can serve as such neurotransmitters. A whole series of peptides having this function are already known (see for example the papers published in the New England Journal of Medicine, Vol. 304, pp. 876-885 and 944-951).

Nowadays a polypeptide called Substance P may be classed among transmitter substances because it has been shown to occur both in nerves transmitting impulses from peripheral parts of the body to the brain (afferents)

and nerves extending from the brain (or peripheral ganglia) to different peripheral organs (efferents).

Substance P is deemed to play an important part in the transmission of pain stimuli from the periphery to the brain. The efferent nerves which contain Substance P are apparently capable of stimulating secretion from endocrine glands and causing vasodilation and contraction of smooth muscle.

Although Substance P was discovered already in 1931 by von Euler and Gaddum, J. Physiol. 72, 74 (1931), the structure of Substance P

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

was not discovered until 1971 by Chang et al, Nature New Biol. 232, 86 (1971).

It has long been known that stimulating effects produced by neurotransmitters can be reduced or suspended. Such blocking of the nervous impulse or its effect can be achieved by substances which are in close chemical relation to the transmitter itself and which therefore react with the sensors (receptors) via which the transmitter substances transmit impulses in the nerve synapses or act upon their targets. A great number of substances are known which have such a blocking (antagonistic) effect on neurotransmitters, although most of them refer to the neurotransmitters in the adrenergic or cholinergic nerves.

Prior Art

It is only in the last few years that one has tried to find antagonists against Substance P. Recently published results of examinations of some antagonists against Substance P have shown that these antagonists have an inhibiting effect on Substance P-induced salivation and on smooth muscle stimulated by Substance P. (See K Folkers et al, Acta Physiol, Scand 1981, 111, 505-506).

It has been suggested that a compound having the structure (D-Pro^2, D-$Trp^{7,9}$) Substance P as referred

to in the present application, is an antagonist against Substance P. The compound in question was shown specifically to block in vivo the Substance P-induced excitation of locus coerulens neurons and was therefore considered to be a CNS antagonist against Substance P.

The works which have so far been published on antagonists against Substance P have solely aimed at finding antagonists, thereby to be able to establish which physiological functions in the body are controlled or affected by Substance P, but any effective drugs have not been developed.

The Invention

It has now been found, very surprisingly, that the substance (D-Pro^2-D-Trp^{7,9}) Substance P as synthetized by us has an inhibiting effect on inflammatory conditions in the body, especially the skin, the upper air-passages, the intestine and the eye.

The present invention therefore relates to the Substance P antagonist (D-Pro^2, D-Trp^{7,9}) Substance P for use in drugs for the prophylactic or therapeutic treatment of diseases released by substance P. In addition, the invention concerns a drug for the prophylactic or therapeutic treatment of inflammations, in particular inflammations in the eye. Furthermore, the invention relates to a method of prophylatically or therapeutically treating diseases released by Substance P, in particular such diseases as inflammations in the skin, the upper air-passages, the intestine and/or the eye. A suitable form for administration of the drug according to this invention is by topical application.

Preparation of the Substance P antagonist according to the invention

The following abbreviations are used:

Met	Methionine
Leu	Leucine
Trp	Tryptophan
Phe	Phenylalanine
Gln	Glutamine
Pro	Proline
Lys	Lysine
Arg	Arginine
DCC	Dicyclohexyl carbodiimide
AC_2O	Acetic anhydride
Py	Pyridine
HONp	p-nitrophenol
Np	Nitrophenyl
Boc	t-butyloxycarbonyl
TFA	Trifluoroacetic acid
DMF	Dimethylformamide
BHA	Benzhydrylamine

($D\text{-}Pro^2$, $D\text{-}Trp^{7,9}$) Substance P having the formula

L-Arg-D-Pro-L-Lys-L-Pro-L-Gln-L-Gln-D-Trp-L-Phe-D-Trp-L-Leu-L-Met-NH_2

is prepared in a manner that is conventional in this particular field, viz. by the solid phase method described by R.B. Merrifield (J. Am. Chem. Soc., 85, (1963), 2149-2154). The solid phase employed is the benzhydrylamine resin described by Monahan et al. (Biochem. Biophys. Res. Com. 48, 1100-1105, (1972)).

The starting materials employed in the preparation were Boc-Arg-Tos, Boc-Gln-ONp and Boc-Leu from Ferring AB, Malmö, Sweden; D-Pro, Pro, D-Trp and Phe from Merck Darmstadt, Federal Republic of Germany; and the benzhydrylamine resin from Beckman Inc.

The t-butyloxycarbonyl protective group (Boc) was coupled to D-Pro, Pro, Phe and D-Trp by means of 2-(t-butyloxycarbonyl oxyimino)-2-phenylacetonitrile (Boc-ON).

(M. Itoh, D. Hagiwara, T. Kamiya, Tetrahedron Letters 4393 (1975)).

The synthesis was conducted by stages, starting from 5 g BHA resin, 0.51 meq. NH_2/g, and the amino acids in the compound according to the invention were coupled according to the following schedule

1	Boc-Met-NH-BHA
2	Boc-Leu-Met-NH-BHA
3	Boc-D-Trp-Leu-Met-NH-BHA
4	Boc-Phe-D-Trp-Leu-Met-NH-BHA
5	Boc-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA
6	Boc-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA
7	Boc-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA
8	Boc-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA
9	Boc-Lys(Z)-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA
10	Boc-D-Pro-Lys(Z)-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA
11	Boc-Arg (Tos)-D-Pro-Lys(Z)-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Met-NH-BHA

After the coupling stages, the peptide was split off from the resin, and after purification of the peptide by gel filtration and column chromatography as well as lyophilization, the required product was obtained which was then analyzed.

HPLC

Column:	µ-Bondapak C 18, (Waters), 2.4x300 mm
Mobile phase:	32% CH_3CN, 68% K-phosphate buffer 0.1 M, ph 3.0
Flow rate:	1.5 ml/min
Detection:	210 nm

In this system, the substance had a retention time of 8.14 min. It gave a single symmetrical shoulderless peak, with an integral of 98.1 area percent.

Thin-layer chromatography

Thin-layer chromatography was run on Merck HPTLC plates (Silica gel 60). Distance run: 15 cm. The substance was uniform in the following solvent system:

$CHCl_3$-conc. NH_3-CH_3OH = 60/20/45	R_f:	0.4
EtOAc-Py-AcOH-H_2O = 5/5/1/3	R_f:	0.78
n-BuOH-EtOAc-AcOH-H_2O = 2/2/1/1	R_f:	0.02
n-BuOH-Py-AcOH-H_2O = 30/3/6/24	R_f:	0.59
2-PrOH-1N AcOH = 2/1	R_f:	0.03

Amino acid analysis

For the analysis, the substance (1 mg) was hydrolized in 1 ml 4 M methane sulphonic acid containing 0.2% tryptamine for 24 h in a sealed tube at 120°C. The amino acid analysis was carried out on a Biotronik-LC-2000 amino acid analyser. For the individual amino acids, the following results were obtained:

Glu: 2.08; Pro: 2.00; Met: 1.05; Leu: 0.99; Phe: 1.01; Arg: 1.06; Trp: 1.9.

The peptide content of the material was 86.7%, based on the triacetate.

Optical rotation

Measuring was effected with a Perkin-Elmer polarimeter. For the measuring, 10 mg of the substance were dissolved in 1 ml methanol, and measuring was carried out at a layer thickness of 10 cm.

Temp. = 25°C.

Thus, there was obtained $\alpha_D^{25^\circ} = -40.1^\circ$.

The effect of (D-Pro^2, D-Trp^{7,9}) Substance P on inflammation

The Substance P antagonist used for the present invention was shown to inhibit or cure inflammation by the following experiment.

Injection of Substance P into the corpus vitrium of a rabbit's eye evokes inflammatory reaction. The inflammatory effects were recorded on the basis of the ensuing miosis and the increase in the amount of protein within the chamber fluid. Th change in the pupillary diameter was measured with a ruler, and the amount of protein in the chamber fluid was estimated by measur-

ing the so-called "light path" which is determined by admitting a luminous ray into the eye and recording the Tyndall phenomenon in the eye chamber which is a consequence of the light-reflecting ability of the protein in the chamber fluid. This reflection phenomenon may be quantized and related to a standard according to a method previously described by Anjou and Krakau (Acta Ophthalmol. 39, 1, 1961).

A similar inflammatory reaction can be evoked by infrared radiation of a restricted area on the iris.

Three rabbits were injected with 30 nmol Substance P antagonist according to the invention in the corpus vitrium of the left eye, and with 0.9% saline in the corpus vitrium of the right eye. After three hours, 3 nmol Substance P were injected in the same localities. One hour later the differences between the pupillary diameters of both eyes were measured, and it was found that the pupillary diameter of the left eye had been reduced by about 0.5 mm, while the pupillary diameter of the right eye had been reduced by about 2.7 mm, and the amount of protein in the chamber fluid in the right eye had increased twice as much as in the left eye. By these experiments, it could be established that the Substance P antagonist according to this invention in itself had but an insignificant effect on the eye.

90 nmol of the Substance P antagonist according to this invention were injected into the corpus vitrium of the left eye (3 rabbits). The right eye was used for control purposes and was given 0.9% saline. After three hours, the irises of both eyes were exposed to infrared radiation. One hour after radiation, the amount of protein in the chamber fluid was measured, and it was found that the so-called light path in the left, treated eye was substantially unchanged, whereas it had been trebled in the right control eye.

Analogous experiments were carried out by administering the Substance P antagonist and the saline in eye

drops, and it was found that the so-called light path was more than twice as large in the control eye than in the treated eye.

The experiments carried out to induce inflammation by infrared radiation also comprised experiments which showed that the minimum amount of recordable effects was 0.9 nmol of the Substance P antagonist in saline, while the minimum dose for maximum effect was 90 nmol in saline. The effect of the antagonist was found to be dose-dependent and rather linear.

To sum up, it can be established that inflammation of the eye, evoked by injection of Substance P into the corpus vitrium or by infrared radiation of the iris, was inhibited or cured by the Substance P antagonist according to this invention which was injected into the corpus vitrium or administered in the form of eye drops.

Preparation of a solution for topical application

The purified substance (D-Pro^2, D-Trp^{7,9}) Substance P which had been kept in lyophilized form, was dissolved in saline to suitable concentration. The solution was filtered under sterile conditions and filled into sterile bottles, whereupon it was used for the experiments described above.

In the above experiments, the Substance P antagonist according to the invention was used dissolved in saline for injection and eye drops, but other forms of administration for topical application by spraying, inhalation, instillation, insufflation and injection are also possible. Furthermore, salves, creams, suppositories, tablets, capsules and granulates can be prepared in which the active constituent consists of the Substance P antagonist according to this invention and the remaining ingredients are conventional inert carriers, diluents, excipients etc.

CLAIMS

1. A Substance P antagonist for use in drugs for the prophylactic or therapeutic treatment of diseases released by Substance P, c h a r a c t e r i s e d in that it consists of (D-Pro^2, D-Trp^{7,9}) Substance P having the formula

L-Arg-D-Pro-L-Lys-L-Pro-L-Gln-L-Gln-D-Trp-L-Phe-D-Trp-L-Leu-L-Met-NH_2

2. A drug for the prophylactic or therapeutic treatment of inflammations, c h a r a c t e r i s e d in that it comprises, as the active substance, (D-Pro^2, D-Trp^{7,9}) Substance P together with an inert carrier or excipient.

3. A drug for the prophylactic or therapeutic treatment of inflammations in the eye, c h a r a c t e r i s e d in that it comprises, as the active substance, (D-Pro^2, D-Trp^{7,9}) Substance P together with an inert carrier or excipient.

4. A drug as claimed in claim 2 or 3, c h a r a c t e r i s e d in that it is available in a form suitable for topical application.

5. A method of prophylactically or therapeutically treating a disease released by Substance P, c h a r a c t e r i s e d by administering a prophylactically or therapeutically active amount of (D-Pro^2, D-Trp^{7,9}) Substance P.

6. A method as claimed in claim 5, c h a r a c t e r i s e d by administering the prophylactically or therapeutically active amount of (D-Pro^2, D-Trp^{7,9}) Substance P by topical application.

7. A method of prophylactically or therapeutically treating inflammations, c h a r a c e r i s e d by administering a prophylactically or therapeutically active amount of (D-Pro^2, D-Trp^{7,9}) Substance P.

8. A method as claimed in claim 7, c h a r a c t e r i s e d by administering the prophylactically

or therapeutically active amount of (D-Pro^{7}, D-Trp^{7,9}) Substance P by topical application.

9. A method of prophylactically or therapeutically treating inflammations in the eye, c h a r a c t e r i s e d by administering a prophylactically or therapeutically active amount of (D-Pro^{2}, D-Trp^{7,9}) Substance P.

10. A method as claimed in claim 9, c h a r a c t e r i s e d by administering the prophylactically or therapeutically active amount of (D-Pro^{2}, D-Trp^{7,9}) Substance P by topical application.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE81/00292

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC 3

C 07 C 103/52, A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC 3	C 07 C 103/52, A 61 K 37/00,02
US Cl	260:8, 112.5; 424:177

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	Acta Physiol Scand. Vol 111, p 505-506, published 1981, FOLKERS K et al, "Chemical design of antagonists of Substance P".	1
A	Chemical Abstracts, Vol 94 (1981), abstract No 203034c, Acta Physiol. Scand. 1981, 111 (3), 381-382 (Eng).	1

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
1982-04-30	1982 -05- 11
International Searching Authority [1]	**Signature of Authorized Officer [20]**
Swedish Patent Office	Elisabeth Carlborg

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 5-10 because they relate to subject matter [12] not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by therapy. Rule 39.

2. [ ] Claim numbers ....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out [13], specifically:

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.